THE VELIGER

The Veliger 27(3):331–335 (January 2, 1985)

An Anesthetic for Internal Operations on the Land Snail *Helix aspersa* Müller

by

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Abstract. An anesthetic solution consisting of 2% magnesium chloride and 0.01% succinylcholine chloride in combination was used to anesthetize *Helix aspersa* for internal operations. A dosage of 0.012 mg of succinylcholine per gram of snail tissue and 2.4 mg/g $MgCl_2 \cdot 6H_2O$ was injected into the hemocoel to produce a quick and pronounced anesthesia with a nearly 100% survival rate. The combined action of the two drugs at low dosages resulted in an anesthesia better than either drug alone at high dosages. At a higher dosage, the new anesthetic solution also worked on *Arion circumscriptus* and may be useful for operations on other land snails.

INTRODUCTION

A LARGE NUMBER OF agents is used for relaxing gastropods. RUNHAM *et al.* (1965) reviewed the effectiveness of a number of narcotizing agents used to relax snails before fixation in an extended position and anesthetics used for relaxing snails prior to operations. Proper relaxation must leave living snails in an extended position and insensitive to touch and to chemicals. Agents that have been tried on snails in recent years include CO_2, hypothermia, dilute formalin, dilute seawater, menthol, propylene phenoxytol, ether, urethane, tricaine (MS-222), Stovaine, Nembutal (see RUNHAM *et al.*, 1965), succinylcholine chloride (BEEMAN, 1968; BURTON, 1975), curare, Novocaine, Xylocaine, methohexital sodium (BEEMAN, 1968), calcium-free seawater, Benzocaine, procaine hydrochloride (Novocaine) (STIRLING *et al.*, 1984), manganese sulfate (YESCOTT & HANSEN, 1976), pentobarbital (MEIER-BROOK, 1976), magnesium chloride (numerous authors, including RUNHAM *et al.*, 1965; TURNER, 1976), Althesin, benzyl alcohol, quinaldine, xylazine (BOURNE, 1984), nicotine, dilute ethanol, and various combinations of these agents. Those reported to be useful for internal operations on gastropods include propylene phenoxytol, magnesium chloride, Nembutal/MS-222 (evaluated by RUNHAM *et al.*, 1965), CO_2 (BAILEY, 1969), Nembutal/$MgCl_2$ (JEPPESEN, 1976), and isotonic $MgCl_2$ (ROBERTS & BLOCK, 1982). BURTON (1975) reported that succinylcholine chloride was useful for anesthetizing *Helix pomatia* Linnaeus for tentacle excision. RUNHAM *et al.* (1965) reported that urethane and ether left snails imperfectly relaxed and was found to be suitable only for external operations, although MALEK & CHENG (1974) recommended both agents with the use of physical retraction.

The effectiveness of many anesthetic agents has been frequently reported to vary widely among species, though a few agents, such as Nembutal and $MgCl_2$, have been used successfully on numerous species.

I have tried various combinations and dosages of $MgCl_2$, Nembutal (sodium 5-ethyl-5-[1-methylbutyl]-barbiturate, or pentobarbital sodium), MS-222 (ethyl m-aminobenzoate methanesulfonate, or tricaine), and succinylcholine chloride in an effort to obtain a quick, injectable anesthetic for internal operations on *Helix aspersa* Müller. Injection of Nembutal and MS-222 did not relax *Helix aspersa* well, so no further trials of MS-222 were conducted. The methods described by JEPPESEN (1976) and BURTON (1975) for *Helix pomatia* gave the most satisfactory results, but were either time-consuming and/or led to unacceptable mortality rates and difficulty during surgery. I report here a quick-acting anesthetic solution with a high survival rate that may also be useful for other large land snails. The results of some preliminary trials of injections of $MgCl_2$, Nembutal, and succinylcholine chloride are presented to facilitate discussion of the effect of the working anesthetic solution.

MATERIALS, METHODS, AND TECHNIQUE

Preliminary Trials

Healthy adult and subadult specimens of *Helix aspersa* obtained from California were used to test various con-

Table 1

The dosage and evaluation of anesthetic solutions on *Helix aspersa*. Dosage is in mg of drug per gram of snail tissue (weight of whole snail minus weight of shell). Evaluation: good (G) for full anesthesia, fair (F) for partial anesthesia adequate for very quick surgery, unsatisfactory (U) for poor relaxation and high irritability of the snail, and no effect (NE) for no response to the injected solution. SChCl = succinylcholine chloride.

Solution	Approximate dosage	Number of snails injected	Evaluation			
			G	F	U	NE
10% $MgCl_2$	12.0 mg/g	11	1	3	7	
5% $MgCl_2$	6.0 mg/g	11		2	9	
2% $MgCl_2$	2.4 mg/g	10			10	
0.25% SChCl	0.30 mg/g	12			12	
0.05% SChCl	0.06 mg/g	13		3	10	
0.01% SChCl	0.012 mg/g	14			14	
0.25% Nembutal	0.30 mg/g	11			11	
0.1% Nembutal	0.12 mg/g	10			10	
2% $MgCl_2$/ 0.1% Nembutal	2.4 mg/g 0.12 mg/g	10			10	
2% $MgCl_2$/ 0.05% SChCl	2.4 mg/g 0.06 mg/g	10	10			
2% $MgCl_2$/ 0.01% SChCl/ 0.005% Streptomycin sulfate	2.4 mg/g 0.012 mg/g 0.006 mg/g	98	98			
0.05% Streptomycin sulfate	0.06 mg/g	9				9

centrations and combinations of $MgCl_2$, Nembutal, and succinylcholine chloride (Table 1). Snails weighing 4 to 7 g were injected through the thin body wall of the upper left region of the head-foot just below the mantle collar (the "nuchal" region). The snails were washed and allowed to crawl around until the shell could be lifted and tilted so that the "nuchal" region was exposed; the injection was then made into the hemocoel. Mild massage of the head-foot facilitated an even distribution of the anesthetic. All the snails of each group received approximately the same dosage. The effectiveness of the solution injected was judged by its ability to: (1) render the snail immobile and unresponsive to pinches on the side of the head-foot, (2) keep the snail completely insensitive for at least 10 min after the injection, and (3) wear off within one or two days. The anesthetic was judged to be good (G) if all these criteria were met, fair (F) if the criteria were met partly with the snail slightly reactive to touch, but relaxed enough for minor external or quick internal surgery, unsatisfactory (U) if none of the criteria were met or if the snail remained very reactive to pinches of the body wall, and with no effect (NE) if the snail showed no change or slowing of movement.

Working Anesthetic Solution and Technique

Ninety-eight healthy adult and subadult specimens of *Helix aspersa* were injected with approximately 0.4 to 0.7 mL of the following solution:

2% $MgCl_2 \cdot 6H_2O$
0.01% succinylcholine chloride (Sigma Chem. Co., St. Louis, Missouri)
0.005% streptomycin sulfate
w/v, in distilled H_2O.

The solution was prepared immediately before use and remained effective for one day. The snails were injected with a volume that delivered an approximate dosage of 0.012 mg of succinylcholine chloride per gram of snail tissue (weight of whole snail minus weight of shell). This amounted to about 0.65 mL for an average-sized snail with a tissue weight of 5.4 g (6.3 g for the whole snail). The volume of anesthetic was roughly proportional to body weight. This seemed reasonable considering the fact that respiration in helicids has been reported to be nearly proportional to weight (see GHIRETTI & GHIRETTI-MAGALDI, 1975). The weight of the snail tissue was calculated from the weight of the whole snail using a regression line previously obtained from 38 specimens of *H. aspersa*: $Y = 0.884X - 0.155$, where Y is tissue weight in grams (weight of whole snail minus weight of shell) and X is weight of the whole snail in grams; $r = 0.975$, Y ranging from 3.5 to 8.0 g. Snails were injected in the manner described above. In order to check for the possible anesthetic effect of the antibiotic streptomycin sulfate, a few snails were injected with a 0.05% streptomycin solution, which was 10 times the concentration in the anesthetic solution. Antibiotics have been reported to have an anesthetic effect in mammals (see GILMAN *et al.*, 1980).

Survival of snails undergoing surgery seemed to depend on retention of body fluids during surgery and on allowing sufficient rest during recuperation. For surgery on the terminal genitalia, the head-foot of the snail was placed on a dissection dish so that the shell lay in a shell-shaped depression in the wax. With the shell lying in a depression, most of the body fluids remained in the visceral hemocoel, and loss of fluid in short operations was usually less than 0.2 mL. The incision was sutured with fine surgical silk using a No. 2 eye type, half-curved needle (George Tiemann & Co., New York). Snails were then placed into clean, dry containers and allowed to estivate for one week. The sutures were removed after this period of estivation. Two snails that appeared dehydrated and ill after surgery were put into containers lined with moist soil and given carrots to feed on for one week before the sutures were removed. The operations performed included excision of parts of the genitals and implantation of tissue from other snails into the hemocoel. The fully recovered snails were kept in soil-lined containers and fed carrots.

Fifteen specimens of *Arion circumscriptus* Johnston were injected to determine the effect of the working anesthetic solution on other snails. Seven of these slugs were cut open and then sutured; the other eight were undisturbed. The slugs were given a dosage of about 0.25 mg succinylcholine per gram body weight, about 20 times the dosage for *Helix aspersa*; this dosage was necessary for complete anesthesia during surgery. The slugs weighed 0.45 g on average and had a maximum body length of 25 mm. Anesthetized slugs were put into containers with moist paper towels until they recovered. The anesthetic solution was also tried as a soaking solution on 10 specimens of *Biomphalaria glabrata* (Say). I observed the state of anesthesia but did not operate on these snails.

RESULTS

Preliminary Trials

The results of the preliminary trials are shown in Table 1. The effect of each solution was generally very uniform, even though the numbers of snails injected in these preliminary trials were small. Snails injected with the $MgCl_2$ solution recovered quickly from its effects—all were able to crawl within 30 min after injection. Snails receiving a 10% solution showed a wide range of reactions; most snails remained sensitive to pinches, though some remained fairly insensitive long enough for quick internal operations. Those receiving a 5% solution writhed when pinched on the side of the head-foot and began to crawl again 15 min after the injection. Those snails receiving a 2% solution never became completely anesthetized and continuously writhed slowly. Only one injection of the 32 snails receiving the $MgCl_2$ solutions resulted in a rating of good (G). Snails injected with Nembutal never became fully anesthetized. Those injected with the 0.25% solution slowly withdrew into the shell or writhed when touched. Those injected with the 0.1% solution writhed slowly and continuously; they appeared to be trying to crawl but seemed unable to coordinate their movements.

Snails injected with different concentrations of succinylcholine chloride showed an odd trend. Those receiving high dosages (0.25%) withdrew their heads after injection and remained highly reactive to light touch thereafter. Those receiving a lower dosage (0.05%) became insensitive to touch for 2 to 4 min after injection, long enough for quick external operations. Snails injected with the lowest dosage (0.01%) were insensitive for only about 1 to 2 min and subsequently recovered. None of the injections of 39 snails receiving the succinylcholine chloride injections was rated as good (G).

Those snails that received a solution of 2% $MgCl_2$/0.01% Nembutal became limp and extended, but writhed when pinched. Snails injected with a solution of 2% $MgCl_2$/0.05% succinylcholine chloride became fully anesthetized long enough for operations. The nine snails injected with 0.05% streptomycin sulfate showed no slowing of movement or narcosis of any kind.

All snails recovered the ability to crawl within 24 h after injection. All snails became reactive to stimuli many hours before they could crawl again, except for those that received $MgCl_2$.

Differences between different dosages for any one drug are not statistically significant because of low sample number and an insufficient number of ranks, but the differences in the effects between the $MgCl_2$/succinylcholine mixture and the pure $MgCl_2$ or pure succinylcholine solutions (all dosages pooled) are significant, since they show almost no overlap in their effects (as evaluated in Table 1).

Working Anesthetic Solution

The working anesthetic solution of 2% $MgCl_2$, 0.01% succinylcholine chloride, and 0.005% streptomycin sulfate gave excellent results during operations on 98 snails. The revival rate after the operation and survival rate after one week were both 100%.

The snails anesthetized by this solution became limp and flaccid within a few seconds after injection. They remained insensitive to pinches and touch for at least 10 min after injection. After this time, the tentacles and labial region first regained sensitivity to stimuli, and snails pinched in these places showed some snout retraction momentarily. More than 50% of the operated snails started to crawl again within 6 h after injection, and all snails had recovered the ability to crawl within a day after the injection.

The combination of the two drugs in the working solution worked better than either drug alone, even at high dosages. A somewhat stronger dosage of succinylcholine (0.05%) when used with $MgCl_2$ (see Table 1) was also effective and may be useful for longer operations.

While under the anesthetic, the snails stopped secreting mucus and the pneumostome stopped its rhythmic opening and closing. During operations, the cut surface of the incision on the body wall curled inward slightly and the region around the incision shrank somewhat. This local response could not be abolished at higher dosages, but it did not cause much difficulty with the surgery and helped reduce loss of blood. Blood loss during surgery, determined by measuring the volume of hemolymph remaining in the dissection dish, remained less than 0.2 mL and averaged 0.05 mL, though the volume of fluid loss would depend on the type of surgery being performed.

Arion circumscriptus injected with the working anesthetic solution at a dosage 20 times that given to *Helix aspersa* became fully anesthetized within a few seconds. The local skin tightening response was more pronounced than in *H. aspersa,* probably due to the greater muscularity of the body wall in the slug. The slugs began recovering from the anesthesia within 30 min after injection. All 15 slugs were able to crawl 2 h after injection.

The anesthetic solution could not be used as a soaking solution on the basommatophoran *Biomphalaria glabrata.* These snails slowly withdrew deep into their shells and would not re-emerge until restored to freshwater. They could not be injected, because they remained too sensitive to touch.

DISCUSSION

The new anesthetic solution reported here appears to work better than other anesthetics reported for *Helix aspersa*. The combination of succinylcholine chloride and $MgCl_2$ acts better than either drug alone, perhaps because of potentiation of succinylcholine by magnesium ion. Of the solutions tried in the preliminary trials, 10% $MgCl_2$, 0.05% succinylcholine chloride, and to a lesser degree 5% $MgCl_2$ appeared to be suitable for quick or external surgery. The contractions that occurred during surgery on snails given these solutions were manageable for a short period immediately after injection. However, contractions of the snails increased blood loss and difficulty of surgery. RUNHAM *et al.* (1965) reported a survival rate of 85% one week after surgery on *Helix aspersa* injected with 10% $MgCl_2$ (I estimate a dosage of about 5.8 mg $MgCl_2$ per gram of snail tissue from their figures). JEPPESEN (1976) reported about an 80% survival rate six months after surgery on *H. pomatia* anesthetized by soaking in a 0.1% Nembutal solution followed by injection of up to 1 mL of 10% $MgCl_2$. BURTON (1975) used a dosage of 0.1 mg to 0.017 mg succinylcholine chloride per snail for tentacle excision; this is a dosage of roughly 0.01–0.17 mg/g, if *H. pomatia* tissue weighs an average of 10 g. I estimate that BEEMAN (1968) used a dosage of 0.013 mg/g of succinylcholine to produce a state of reversible narcosis in *Aplysia*. These dosages are comparable to or higher than the dosages of these drugs in the working anesthetic used on *H. aspersa*—0.012 mg/g succinylcholine, 2.4 mg/g $MgCl_2$.

The combination of $MgCl_2$ and succinylcholine produces a pronounced anesthesia. The mechanism for this action in *Helix aspersa* is unknown. However, it is known that in man and cats magnesium ion potentiates the effect of succinylcholine and other muscle relaxants (MORRIS & GIESECKE, 1968; GIESECKE *et al.*, 1968; GHONEIM & LONG, 1970). Magnesium ion has been demonstrated to reduce the release of acetylcholine at the neuromuscular junction in vertebrates (DEL CASTILLO & ENGBAEK, 1954) in a manner similar to that produced by lack of calcium ion (see HUBBARD, 1973). Succinylcholine is an antagonist to acetylcholine in neurons of the subesophageal ganglia of *Helix aspersa* (WALKER & HEDGES, 1967) and is known to produce a neuromuscular block in humans and vertebrates by blocking the action of acetylcholine on the postsynaptic membrane of the neuromuscular junction (see GILMAN *et al.*, 1980). Concurrent application of the two drugs produces an additive effect (GIESECKE *et al.*, 1968; GHONEIM & LONG, 1970). In contrast, I could find no indication of an increase in effect when Nembutal (a barbiturate) and $MgCl_2$ were used together. In humans, barbiturates are hypnotics that work on the central nervous system and have almost no peripheral effect (see GILMAN *et al.*, 1980). I have used a combination of Nembutal, $MgCl_2$, and succinylcholine chloride injected immediately after mixing for operations on more than 250 *H. aspersa*, with a survival rate of nearly 100%, but such a combination was no more effective than the solution not containing the Nembutal.

The interaction between magnesium ion and succinylcholine may be undesirable in humans, but it may be useful to investigators working on snails. The use of agents with similar action may explain the effectiveness of the narcotizing technique reported by STIRLING *et al.* (1984). Their sequential use of calcium-free seawater, $MgCl_2$, and procaine hydrochloride (Novocaine) on veligers may affect nerve endings. In vertebrates, calcium ion functions to release acetylcholine at the synapse and is antagonized by magnesium (see HUBBARD, 1973), and procaine makes nerves less permeable to ions.

The test of the new anesthetic on *Biomphalaria glabrata* indicates that the anesthetic cannot be used as a soaking solution and probably must be injected to anesthetize the snail and simultaneously extend the body from the shell. Extension of the head-foot from the shell is important in operations on shelled gastropods. In slug forms, anesthesia may not be necessary, provided that physical immobilization is used. HARLEY & HARLEY (1973) operated on large *Aplysia californica* without anesthesia and obtained a good survival rate if the weight loss during the operation was kept below 15% of the pre-operative value. A good anesthetic must leave the snail well extended from the shell and prevent major contraction during surgery to reduce blood loss. The successful use of the new anesthetic on *Arion circumscriptus* indicates that the anesthetic solution might be used successfully on other land snails.

ACKNOWLEDGMENTS

I thank Dr. Alex Tompa for suggesting succinylcholine chloride as an anesthetic and in supporting and assisting my research on *Helix*. I am grateful to Dr. James Cather for providing the Nembutal. I thank Dr. A. Tompa and John Petranka for reading and making useful comments on the manuscript. My work on *Helix* has been supported in part by a grant from the Hawaiian Malacological Society and grants from the University of Michigan.

LITERATURE CITED

BAILEY, T. G. 1969. A new anesthetic technique for slugs. Experientia 25:1225.

BEEMAN, R. D. 1968. The use of succinylcholine and other drugs for anesthetizing or narcotizing gastropod mollusks. Pubbl. Staz. Zool. Napoli 36:267–270.

BOURNE, G. B. 1984. Anesthetic methods for the moon snail *Polinices lewisii*. Veliger 26:327–329.

BURTON, R. F. 1975. A method of narcotizing snails (*Helix pomatia*) and cannulating the haemocoel and its application to a study of the role of calcium in the regulation of acid-base balance. Comp. Biochem. Physiol. 52A:483–485.

DEL CASTILLO, J. & L. ENGBAEK. 1954. The nature of the neuromuscular block produced by magnesium. J. Physiol. 124:370–384.

Ghiretti, F. & A. Ghiretti-Magaldi. 1975. Respiration. Pp. 33–52. *In:* V. Fretter & J. Peake (eds.), Pulmonates, Vol. 1. Academic Press: New York.

Ghoneim, M. M. & J. P. Long. 1970. The interaction between magnesium and other neuromuscular blocking agents. Anesthesiology 32:23–27.

Giesecke, A. H., R. E. Morris & C. R. Stephen. 1968. Of magnesium, muscle relaxants, toxemic parturients, and cats. Anesth. Analg. 47:689–695.

Gilman, A. G., L. S. Goodman & A. Gilman. 1980. The pharmacological basis of therapeutics. 6th edition. The Macmillan Co.: New York.

Harley, P. & E. Harley. 1973. A technique for cutting or accessing nerves and connectives in *Aplysia californica.* Comp. Biochem. Physiol. 45A:389–392.

Hubbard, J. I. 1973. Microphysiology of vertebrate neuromuscular transmission. Physiol. Rev. 53:674–723.

Jeppesen, L. L. 1976. The control of mating behaviour in *Helix pomatia* L. (Gastropoda: Pulmonata). Anim. Behav. 24:175–290.

Malek, E. A. & T. C. Cheng. 1974. Medical and economic malacology. Academic Press: New York.

Meier-Brook, C. 1976. An improved relaxing technique for mollusks using pentobarbital. Malacol. Rev. 9:115–117.

Morris, R. & A. H. Giesecke. 1968. Potentiation of muscle relaxants by magnesium sulfate therapy in toxemia of pregnancy. South. Med. J. 61:25–28.

Roberts, M. H. & G. D. Block. 1982. Dissection of circadian organization of *Aplysia* through connective lesions and electrophysiological recording. J. Exp. Zool. 219:39–50.

Runham, N. W., K. Isarankura & B. J. Smith. 1965. Methods for narcotizing and anesthetizing gastropods. Malacologia 2:231–238.

Stirling, P., C. Little, M. C. Pilkington & J. B. Pilkington. 1984. Technique for narcotizing and fixing veliger larvae of *Amphibola crenata.* Veliger 26:229–232.

Turner, R. D. 1976. Fixation and preservation of molluscan zooplankton. Monogr. Oceanogr. Method. No. 4:290–300.

Walker, R. J. & A. Hedges. 1967. The effect of cholinergic antagonists on the response to acetylcholine, acetyl-β-methylcholine and nicotine of neurones of *Helix aspersa.* Comp. Biochem. Physiol. 23:977–989.

Yescott, R. E. & E. L. Hansen. 1976. Effect of manganese on *Biomphalaria glabrata* infected with *Schistosoma mansoni.* J. Invert. Path. 28:315–320.